REMARKS

Entry of the claim amendments is respectfully requested. Claims 3, 8, 17, and 19 are pending. Independent claims 3 and 17 have been amended to incorporate the limitations in dependent claims 14 and 21, respectively, that the ruminant is a bovine animal. Claims 8 and 19 have been amended to recite that administration of the *Escherichia coli* is for 10-13 days, support for which is found on page 9, line 15 of the present specification. Claims 14 and 21 have been canceled. No new matter has been added.

Claims 3, 8, 14, 17, 19, and 21 are rejected under 35 U.S.C. §112, second paragraph as being indefinite. Claims 14 and 21 have been canceled. With respect to claim 8, the Examiner contends that the claim is vague and indefinite in that the time period encompassed by "at least about 10 days" is unclear. Applicant submits that the rejection has been obviated by replacing the term "at least about 10 days" with "for 10 to 13 days." The current specification clearly states that DSM 6601 was "independent of the weight and age of the animal and was administered orally, specifically for a duration of 10-13 days after birth." (*See* p. 9, lns. 14-15). Thus, the specification teaches that the animals can be treated beginning immediately after birth or sometime soon thereafter for a period of 10 to 13 days. As such, claim 8 is not indefinite.

With regard to claims 3 and 17, the Examiner contends that the claims are vague and indefinite in that the "therapeutically effective amount" required to "prevent" diarrhea or to "prevent" intestinal colonization in all subjects for all conditions is not set forth with sufficient particularity in the as filed written disclosure. The Examiner also contends that the period of "preventing" is also unclear, inquiring whether the prevention is during administration.

Applicant respectfully traverses this ground of rejection. As noted in Applicant's March 26, 2004 Amendment, MPEP § 2173.05(c) states that the phrase "effective amount" is not indefinite if those skilled in the art would be able to determine from the written description what an effective amount is. The current specification clearly discloses that the objective of the current invention is to prevent and treat diarrhea in bovine animals using DSM 6601. As such, an "effective amount" is an amount of DSM 6601 that achieves these objectives. Applicant's method almost completely prevented the occurrence of diarrhea due to fungi colonization, as compared to animals not treated with DSM 6601. This prevention extended for much longer than the 10-13 days of treatment. In fact, in some instances, prevention was indicated up to almost 10 weeks following treatment in some instances. (*See* p. 9 lns. 11-13). Moreover, the terms "preventing" and "treating" are clearly outlined on page 10, 2nd paragraph of the present specification stating that:

> "[T]he occurrence of diarrhea due to gastroenteritides in suckling calves was prevented almost completely by the preventive and therapeutic application of a suspension of E. coli DSM 6601".

Thus, it should be clear that the term "treatment" of a disease also comprises prevention of said disease, as the comparable or identical dosages of the active agent is given. Further, the duration or period of the preventive application may be clearly derived from pages 9-10 of the present specification, since "administration for 10 to 13 days" should be read in the context of the above paragraph.

Moreover, the term "therapeutically effective amount" should be read in context with the examples set forth in the specification. On pages 7 through 9, the occurrence of diarrhea in suckling calves (up to 100%) and death (up to 17.4%) mediated

by yeast infection (specifically *Candida glabrata*) is described. In the following section, *E. coli* DSM 6601 is discussed as being administered to "newborn calves" to prevent development of the disease. A total of 174 calves were treated and only 4 (a mere 2.3%) showed symptoms of diarrhea. Thus, in this example, the "therapeutically effective amount" was an amount that yielded an approximately 97% success rate in preventing cases of morbidity and mortality in the treated individuals.

Further, determining the concentration of DSM 6601 necessary to achieve the results is well within the skilled artisan's knowledge and therefore the specification is definite in this regard. Applicant submits, however, that it would be assumed that *E. coli* suspensions with typical bacterial concentrations (counts/ml) would be used by those of ordinary skill in the art to treat the newborn calves. For example, the references discussed on page 5 of the specification refer to the use of Mutaflor in veterinary medicine as early as the 1950's. At the time of the invention, concentrations were typically 10^8-10^9 viable *E. coli* strain Nissle 1917/ml (synonymous with DSM 6601), as shown in the annexed sheets of Mutaflor suspensions from ARDEYPHARM (November 1997) and from an analysis certificate carried out by PHARMA ZENTRALE (Ponsocol, 10^8 to 10^9 viable *E. coli* strain Nissle 1917/DSM 6601/ml). This is further supported by the references cited by the Examiner, which also teach the use of Mutaflor and/or the use of 10^8-10^9 CFU/ml. (*See, e.g., Hockertz,* p. 794; *Lodinova-Zadnikova et al.,* p. 225; and DE 196 37 936, p. 12 of translation.)

In addition, the specification itself teaches that the suspension of living *E. coli* DSM 6601 was administered in an amount of 15 ml per calf per day, <u>independent</u> of the weight and age of the animal. Applicant therefore submits that the specification need not specifically indicate the concentration of *E. coli*, and that one of ordinary skill in the art would

plainly be able to determine from the description what a therapeutically effective amount is. Reconsideration and withdrawal of this ground of rejection are therefore respectfully requested.

Claims 3, 8, 14, 17, 19 and 21 are rejected under 35 U.S.C. §103(a) as being obvious over *Hockertz* taken with *Lodinova-Zadnikova et al.* and DE 196 37 936. Claims 14 and 21 have been canceled. Claims 3, 8, 17, and 19 provide methods for treating and preventing fungi-induced diarrhea in ruminants by the oral administration of *E. coli* strain DSM 6601.

With respect to *Hockertz*, the reference pertains to the immuno-modulatory effect of orally administered *E. coli* strain DSM 6601. Specifically, certain inbred mice were given a single, intravenously administered dosage of *Listeria monocytogenes* or *Candida* 24 hours after having orally received a single dose of *E. coli* DSM 6601. It is reported that pathogen counts found in the respective organs, namely liver and spleen, may be ameliorated by a single previous *E. coli* challenge.

Significantly, the reference neither describes gastrointestinal infection of bacterial or fungus pathogens at all, nor does it note any gastrointestinal complications like diarrhea. Additionally, the reference does not mention bovine animals or ruminants.

It should be noted that in the mouse model system of *Hockertz*, intravenously administered *Listeria* and/or *Candida* induces a systemic infection of the blood system and certain organs, but without effecting the gastrointestinal tract. Significantly, infection of the gastrointestinal tract by *Listeria* and/or *Candida* and associated diarrhea does not occur in this model. By contrast, *Candida* infection of the gut only occurs orally, and its effects are limited to the gut. Accordingly, diarrhea caused by *Candida* does not involve any systemic pathological effects. The model used by *Hockertz*,

therefore, does not allow any conclusion that a single orally administered dose of *E. coli* DSM 6601 may be suitable to treat a potential *Candida* infection of the gut of the mice (or other mammals) with reasonable expectation of success. Moreover, to Applicant's knowledge, there is no report available, either in the field of veterinary or of human medicine, reporting a successful treatment of diarrhea by a single administration of a probiotic such as *E. coli* DSM 6601.

With respect to *Lodinova-Zadnikova et al.*, this reference relates to a clinical study using *E. coli* DSM 6601 in newborn babies to prevent or reduce colonization of the gastrointestinal tract by pathogenic bacteria. It is reported that a five times prophylactic administration of *E. coli* DSM 6601 on five consecutive days reduced pathogenic bacterial load in the gastrointestinal tract of the babies as revealed by microbiological analysis. Significantly, the reference does not mention use of *E. coli* in the treatment of diarrhea caused by *fungi* in humans. Additionally, the reference does not describe bovine animals or other ruminants. Further, neither a bacterial diarrhea was treated nor one induced by the fungi.

Applicant submits that bacteria and fungi are biologically, taxonomically and evolutionarily differing organisms, as discussed in detail on page 3 of the present specification. Bacteria belong to the kingdom of prokaryotes while fungi belong to that of eukaryotes. These two organisms differ not only in their organization, *e.g.* having a real nucleus and organelles, but are also characterized in that bacterial pathogens may be combated by antibiotics whereas pathogenic fungi are to be treated with anti-mycotic agents. Antiobiotics attack cell structures typical for bacteria, while anti-mycotic agents act against structures typical for fungi. Medicaments of both classes are therefore not interchangeable in the treatment of bacterial and fungal infection, respectively.

Thus, the anti-bacterial activity shown in *Lodinova-Zadnikova et al.* of viable *E. coli* DSM 6601 cannot be transferred to conditions involving fungal infections.

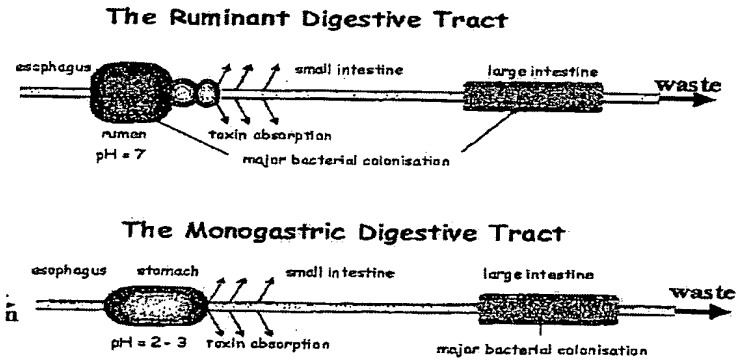
With respect to DE 196 37 936, Applicant notes that it has made a claim for foreign priority benefits under 35 U.S.C. §365 based on DE 197 51 907, filed November 22, 1997, which predates the April 9, 1998 publication date of DE 196 37 936. As noted in Applicant's Amendment dated March 26, 2004, Applicant submitted a certified English translation of priority document DE 197 51 907. It appears, however, that the translation is not of record. Applicant therefore resubmits the certified the English translation herewith. As the current application and the priority document share identical specifications, DE 196 37 936 cannot anticipate the claimed invention.

As the present specification makes clear, the problem underlying the present invention is solved by a method for treating and preventing diarrhea in bovine animals and ruminants by the administration of viable *E. coli* DSM 6601. After all existing therapeutic options had been exhausted, the occurrence of diarrhea due to gastroenteritides in suckling calves was prevented and shown to be effective in 97% of the calves. (*See* p. 9, 2nd paragraph - p. 10, 2nd paragraph).

As discussed above, none of the prior art documents teach the use of *E. coli* DSM 6601 to treat diarrhea caused by pathogenic fungi in bovine animals or other ruminants. From *Hockertz*, one of ordinary skill in the art has been taught to use a single, orally administered *E. coli* strain DSM 6601 challenge to reduce an induced systemic infection of *Candida* or *Listeria monocytogenes* in a mouse. This single, orally administered challenge of *E. coli* twenty-four (24) hours before systemic infection is reported to ameliorate the pathogen count in infected organs such as the liver and the spleen. However,

as noted above, infection of the gastrointestinal tract by *Listeria* and/or *Candida* infection of the gut occurs only in this model. In contrast, *Candida* infection of the gut occurs only orally, and its effects are limited to the gut. Accordingly, Applicant submits that diarrhea caused by *Candida* does not involve any systemic pathological effects.

Moreover, as the Examiner acknowledges and as may be drawn from general knowledge, a mouse is not a human nor is it a bovine animal or other ruminant. This is reflected by the great differences seen with respect to intestinal anatomy and functions between monogastric animals, including humans and ruminants.



Mice (and man) are born monogastric animals. Bacterial colonization of the monogastric digestive tract occurs in the large intestine and in the ceacum. In contrast, cattle are born herbivores and, therefore, their intestinal tract is organized in a completely different way, having a stomach which consists of several compartments. Here, the rumen is the main fermentation vat where billions of micro-organisms attack and break down the relatively indigestible feed.

Not only with respect to their intestinal tract do rodents and ruminants differ but also in their biology. The differences in the natural habitat, in food specialization, in reproduction and in metabolism are enormous, as is the

development of their immune system. Examining the immunological state of an animal, the age (the developmental stage) of the animal to be tested has to be considered, too. Also, susceptibility to infectious agents greatly differs among different species. It is also noted that with respect to the experiments done by *Hockertz,* 6 to 8 week old mice (not neonatal mice) were used. At this age, foodstuff and digestion is that of adult mice, *i.e.* the gastrointestinal tract is fully developed and cannot be compared with the gastrointestinal tract of newborn calves.

In view of the above, *Hockertz's* teaching that an oral dose of *E. coli* DSM 6601 has an effect on the systemic response to a systemic infection does not give any information which reasonably could lead to the teaching or suggestion of the present invention. Thus, *Hockertz* cannot render obvious the present invention.

In addition, the combination of *Hockertz* and *Lodinova-Zadnikova et al.* does not render obvious the present invention. According to *Lodinova-Zadnikova et al.,* one of ordinary skill in the art has been taught to use *E. coli* strain DSM 6601 to prevent or reduce infection of the gastrointestinal tract of newborn babies by pathogenic bacteria. However, as discussed above, and in view of the failure of *Lodinova-Zadnikova et al.* to either mention fungal infections or fungi-mediated diarrhea, *Lodinova-Zadnikova et al.* alone does not render obvious the present invention.

Additionally, with respect to the results and the information taught by *Hockertz,* the same results are not comparable to the results of the clinical study on newborn babies in *Lodinova-Zadnikova et al.,* since newborn human babies were colonized by the probiotic *E. coli* strain DSM 6601 via oral inoculation starting from directly after birth. Since *Lodinova-Zadnikova et al.* fail to discuss fungal gut-associated

infection and ruminants, the skilled person could not supplement the missing information of *Hockertz* with the lacking data from *Lodinova-Zadnikova et al.* to reach at the subject matter of the present invention. As such, *Lodinova-Zadnikova et al.* does not, alone or in combination, render the claimed invention obvious.

With regard to DE 196 37 936, as discussed above, Applicant notes that it has made a claim for foreign priority benefits under 35 U.S.C. §365 based on DE 197 51 907, filed November 22, 1997, which predates the April 9, 1998 publication date of DE 196 37 936. As such, DE 196 37 936 cannot, alone or in combination, be used to render the claimed invention obvious.

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue.

If, however, for any reason the Examiner does not believe that such action can be taken at this time, it is respectfully requested that she telephone Applicant's attorney at (908) 654-5000 in order to overcome any additional objections which she might have.

If there are any additional charges in connection with this requested amendment, the Examiner is authorized to charge Deposit Account No. 12-1095 therefor.

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Respectfully submitted,

By_____

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